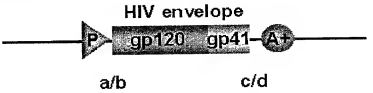
METHODS

Envelope Expression Vector: pHIVenv



HIV-1 Expression Vector: pHIVlucΔU3

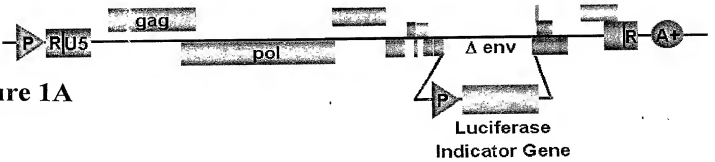


Figure 1A

PhenoSense HIV Entry Cell Assay

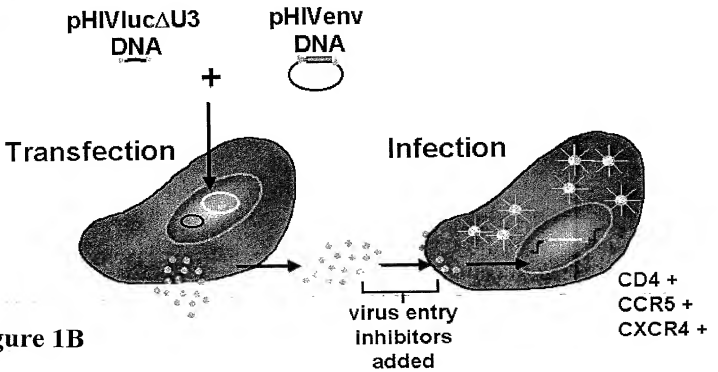


Figure 1B

Figure 2

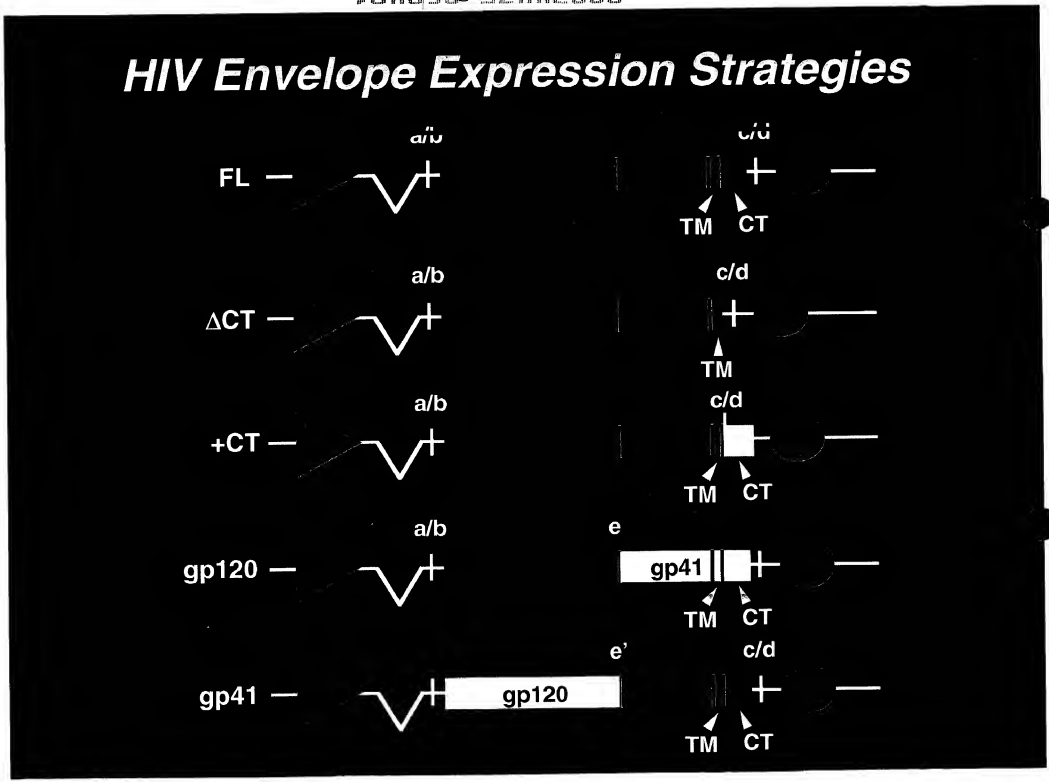
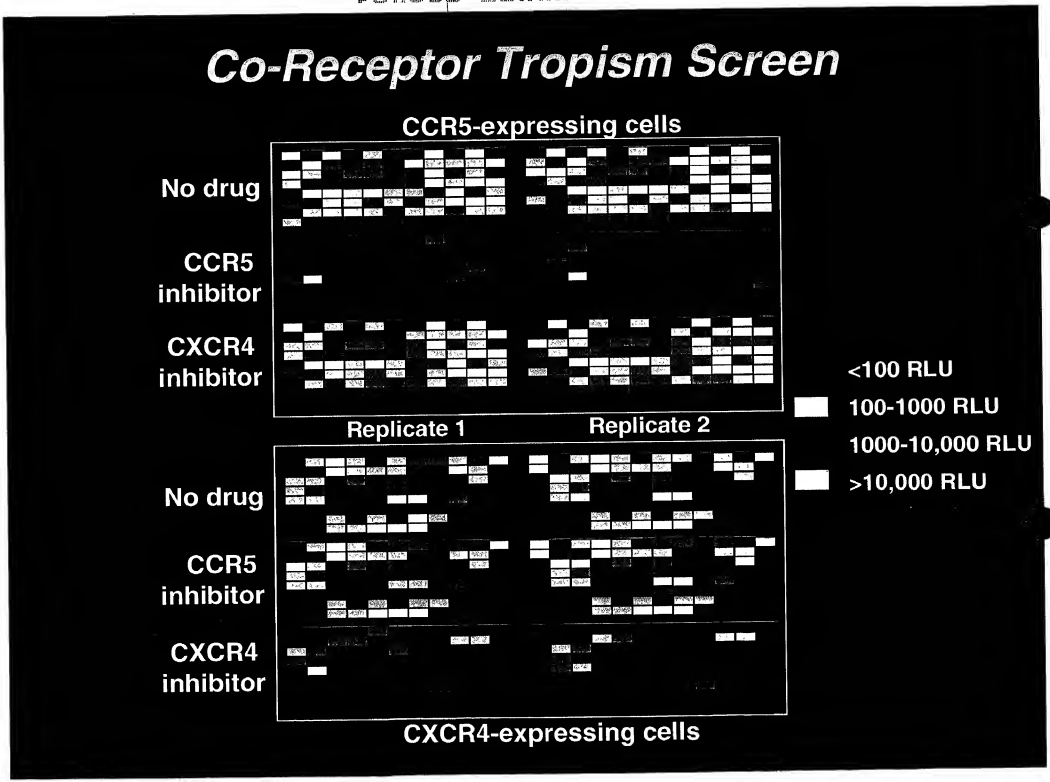

HIV Envelope Expression Strategies
FL
a/b
c/d
TM
CT
ΔCT
a/b
c/d
TM
+CT
a/b
c/d
TM
CT
gp120
a/b
e
gp41
TM
CT
gp41
e'
gp120
c/d
TM
CT

Figure 3A


Co-Receptor Tropism Screen
CCR5-expressing cells
No drug
CCR5 inhibitor
CXCR4 inhibitor
Replicate 1
Replicate 2
No drug
CCR5 inhibitor
CXCR4 inhibitor
CXCR4-expressing cells
<100 RLU
100-1000 RLU
1000-10,000 RLU
>10,000 RLU

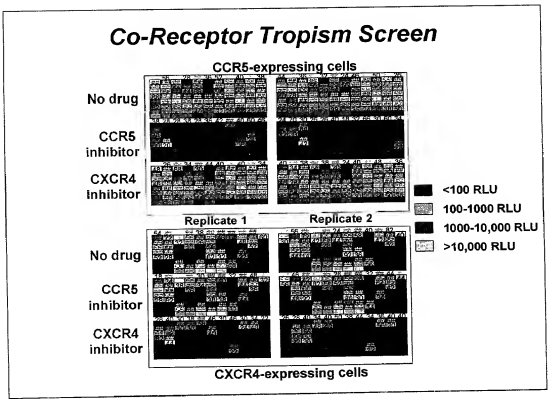
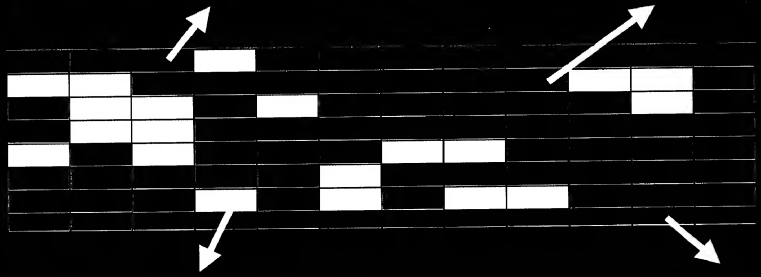


Figure 3A. Co-receptor Tropism Screening Assay

In this embodiment, the assay is performed using two cell lines. One cell line expresses CD4 and CCR5 (top six panels). The other cell line expresses CD4 and CXCR4 (bottom six panels). The assay is performed by infecting cells with a large number of recombinant virus stocks derived from cells transfected with pHIVenv and pHIVlucΔU3 vectors. Tthe example shown represents the analysis of 96 viruses formatted in a 96 well plate. Infections are performed in the absence of drug (no drug), or in the presence of a drug that preferentially inhibits either R5 tropic (CCR inhibitor) or X4 tropic (CXCR4 inhibitor) viruses. Co-receptor tropism is assessed by comparing the amount of luciferase activity produced in each cell type, both in the presence and absence of drug (see Figure 3B for interpretation of assay results).

Figure 3B

Co-Receptor Tropism Assay Interpretation



3

	R5 cells	X4 cells	R5:X4
no drug			
R5 inhibitor			
X4 inhibitor			
%inhib by R5 inhibitor			
%inhib by X4 inhibitor			

21

	R5	X4	R5:X4
no drug			
R5 inhibitor			
X4 inhibitor			
%inhib by R5 inhibitor			
%inhib by X4 inhibitor			

DUAL or MIXED

NON-VIABLE

active RLU limit: 100

tropism ratio limit: 5

76

	R5	X4	R5:X4
no drug	14,982	12,020	1
R5 inhibitor	111	10,839	
X4 inhibitor	8,580	3,384	
%inhib by R5 inhibitor	99	10	
%inhib by X4 inhibitor	43	72	

95

	R5	X4	R5:X4
no drug	43	42	
R5 inhibitor			
X4 inhibitor			
%inhib by R5 inhibitor			
%inhib by X4 inhibitor			

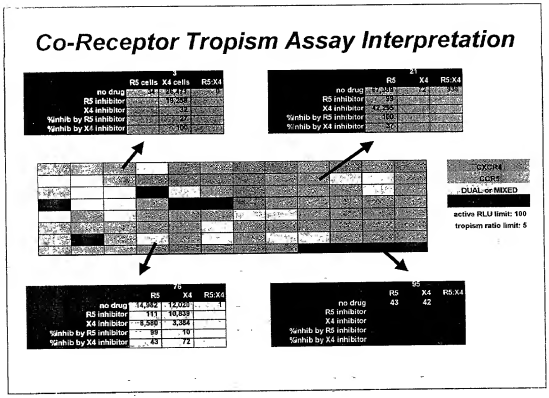


Figure 3B

Determining co-receptor tropism.

In this embodiment, the results of the assay are interpreted by comparing the ability of each sample virus to infect (produce luciferase activity) in cells expressing CD4/CCR5 (R5 cells) or cells expressing CD4/CXCR4 (X4 cells). The ability of a CCR5 or CXCR4 inhibitor to specifically block infection (inhibit luciferase activity) is also evaluated.

X4 tropic viruses (green panels)- infect X4 cells but not R5 cells. Infection of X4 cells is blocked by the CXCR4 inhibitor .

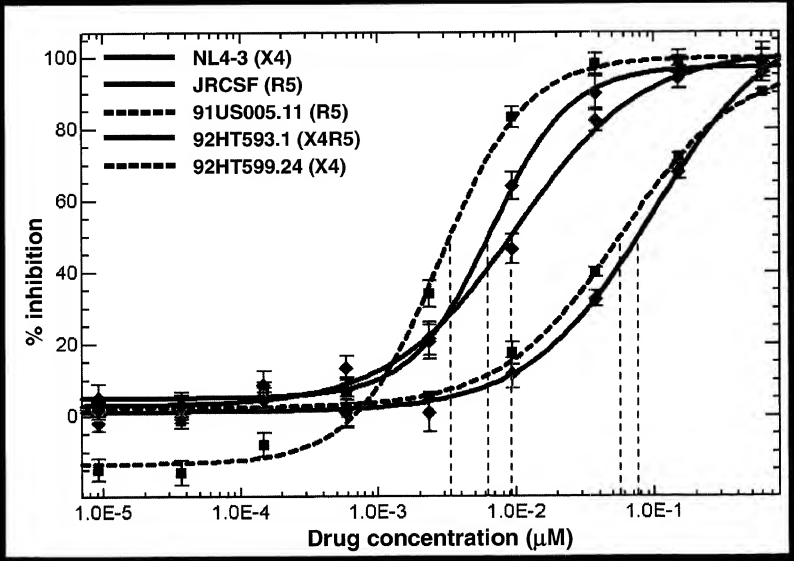
R5 tropic viruses (blue panels)- infect R5 cells but not X4 cells. Infection of R5 cells is blocked by the CCR5 inhibitor .

Dual tropic or X4/R5 mixtures (yellow panels)- infect X4 and R5 cells. Infection of R5 cells is blocked by the CCR5 inhibitor and infection of X4 cells is blocked by the CXCR4 inhibitor .

Non-viable viruses (red panels)- do not replicate in either X4 or R5 cells.

Figure 4A

Entry Inhibitor Susceptibility: Fusion Inhibitor



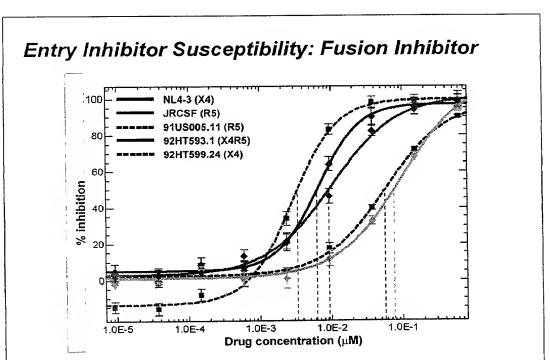


Figure 4A.

Measuring Entry Inhibitor Susceptibility: Fusion Inhibitor

In this embodiment, susceptibility to the fusion inhibitor T-20 is demonstrated. Cells expressing CD4, CCR5 and CXCR4 were infected in the absence of T-20 and over a wide range of T-20 concentrations (x-axis log10 scale). The percent inhibition of viral replication (y-axis) was determined by comparing the amount of luciferase produced in infected cells in the presence of T-20 to the amount of luciferase produced in the absence of T-20. R5 tropic, X4 tropic and dual tropic viruses were tested. Drug susceptibility is quantified by determining the concentration of T-20 required to inhibit 50% of viral replication (IC50, shown as vertical dashed lines). Viruses with lower IC50 values are more susceptible to T-20 than viruses with higher IC50 values.

NL4-3: well-characterized X4 tropic strain

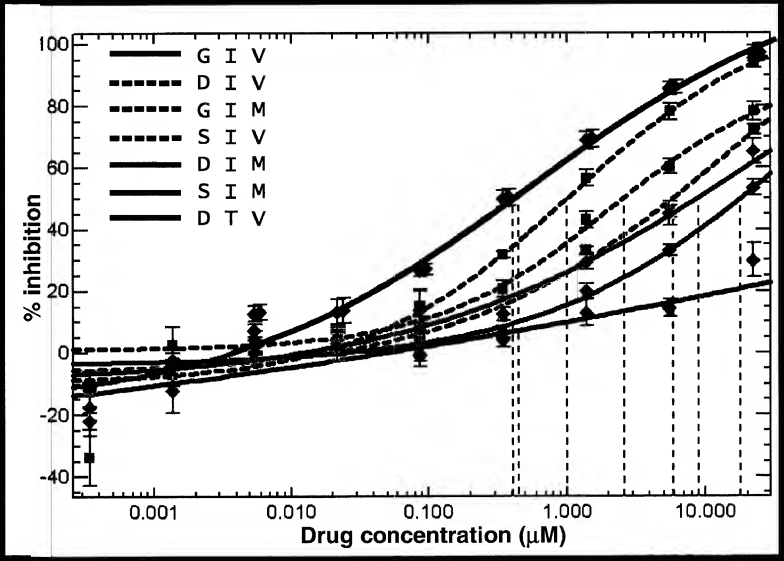
JRCSF: well-characterized R5 tropic strain

91US005.11: R5 tropic isolate obtained from the NIH AIDS Research and Reference Reagent Program (ARRRP)

92HT593.1: Dual tropic (X4R5) isolate obtained from the NIH ARRRP.

Figure 4B

Reduced Susceptibility: Fusion Inhibitor



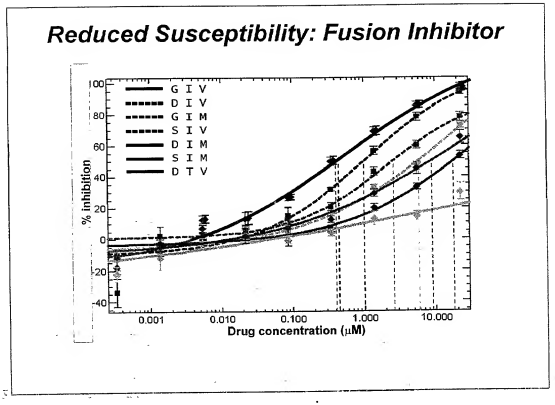


Figure 4B.

Measuring Entry Inhibitor Susceptibility: Drug Resistance Mutations

In this embodiment, reduced susceptibility to the fusion inhibitor T-20 conferred by specific drug resistance mutations in the gp41 envelope protein is demonstrated. Cells expressing CD4, CCR5 and CXCR4 were infected in the absence of T-20 and over a wide range of T-20 concentrations (x-axis log10 scale). The percent inhibition of viral replication (y-axis) was determined by comparing the amount of luciferase produced in infected cells in the presence of T-20 to the amount of luciferase produced in the absence of T-20. Isogenic viruses containing one or two specific mutations in the gp41 transmembrane envelope protein were tested (highlighted in red in the figure legend). Drug susceptibility is quantified by determining the concentration of T-20 required to inhibit 50% of viral replication (IC50, shown as vertical dashed lines). Viruses with lower IC50 values are more susceptible to T-20 than viruses with higher IC50 values.

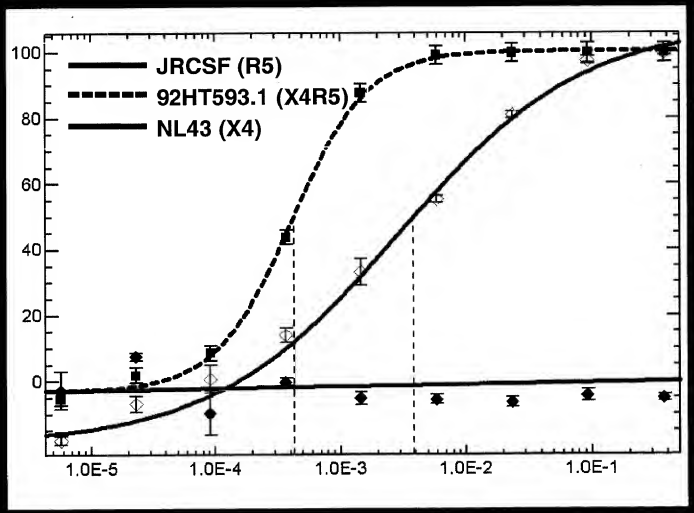
No mutation (wildtype sequence): GIV

Single mutations: GIV, DIM, SIV

Double mutations: DIM, SIM, DTV

Figure 5A

Entry Inhibitor Susceptibility: CCR5 Inhibitor



Drug: R5 Inhibitor
Cell: CD4/CCR5

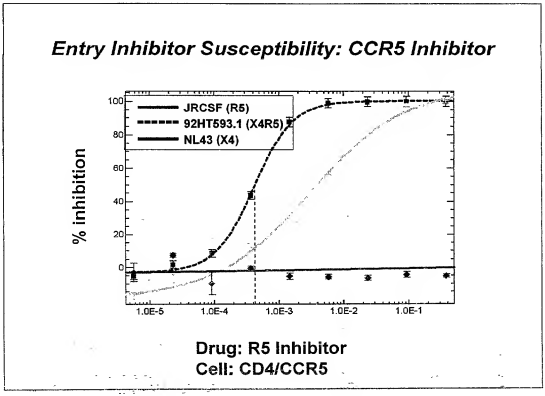


Figure 5A.

Measuring Entry Inhibitor Susceptibility: CCR5 Inhibitor

In this embodiment, susceptibility to a CCR5 inhibitor (merck compound) is demonstrated. Cells expressing CD4 and CCR5 (R5 cells) were infected in the absence of the CCR5 inhibitor and over a wide range of CCR5 inhibitor concentrations (x-axis log10 scale). The percent inhibition of viral replication (y-axis) was determined by comparing the amount of luciferase produced in infected cells in the presence of CCR5 inhibitor to the amount of luciferase produced in the absence of CCR5 inhibitor. R5 tropic, X4 tropic and dual tropic viruses were tested. Drug susceptibility is quantified by determining the concentration of CCR5 inhibitor required to inhibit 50% of viral replication (IC50, shown as vertical dashed lines). Viruses with lower IC50 values are more susceptible to the CCR5 inhibitor than viruses with higher IC50 values. The X4 tropic virus did not infect the R5 cells.

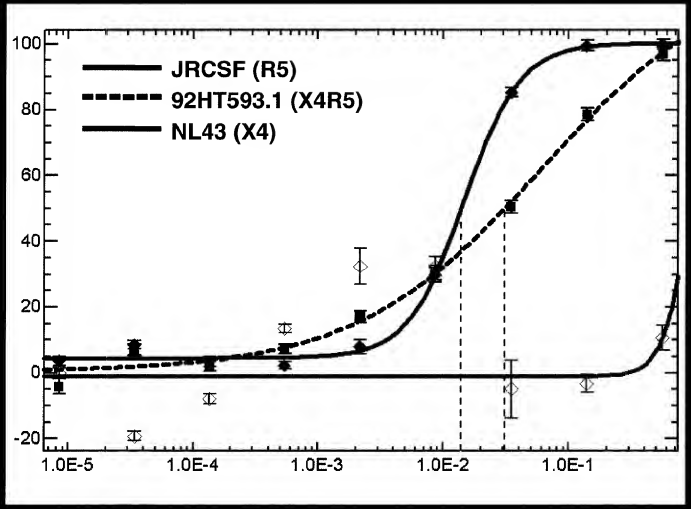
NL4-3: well-characterized X4 tropic strain

JRCSF: well-characterized R5 tropic strain

92HT593.1: Dual tropic (X4R5) isolate obtained from the NIH ARRRP.

Figure 5B

Entry Inhibitor Susceptibility: CXCR4 Inhibitor



Drug: X4 Inhibitor
Cell: CD4/CXCR4

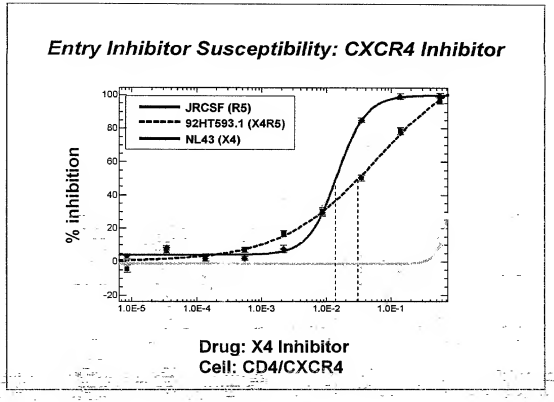


Figure 5B.

Measuring Entry Inhibitor Susceptibility: CXCR4 Inhibitor

In this embodiment, susceptibility to a CXCR4 inhibitor (AMD3100) is demonstrated. Cells expressing CD4 and CXCR4 (X4 cells) were infected in the absence of the CXCR4 inhibitor and over a wide range of CXCR4 inhibitor concentrations (x-axis log10 scale). The percent inhibition of viral replication (y-axis) was determined by comparing the amount of luciferase produced in infected cells in the presence of CXCR4 inhibitor to the amount of luciferase produced in the absence of CXCR4 inhibitor. R5 tropic, X4 tropic and dual tropic viruses were tested. Drug susceptibility is quantified by determining the concentration of CXCR4 inhibitor required to inhibit 50% of viral replication (IC50, shown as vertical dashed lines). Viruses with lower IC50 values are more susceptible to the CCR5 inhibitor than viruses with higher IC50 values. The R5 tropic virus did not infect the X4 cells.

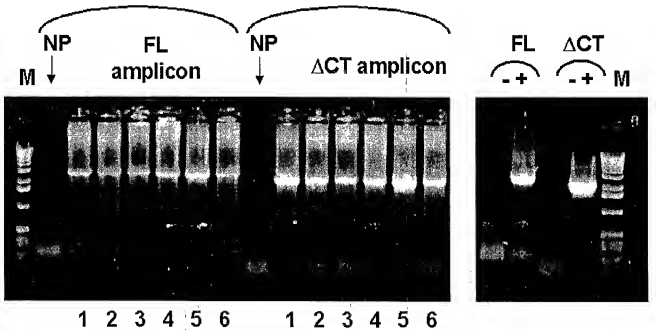
NL4-3: well-characterized X4 tropic strain

JRCSF: well-characterized R5 tropic strain

92HT593.1: Dual tropic (X4R5) isolate obtained from the NIH ARRRP.

Figure 6

2. Envelope Sequence Amplification



1. R5
2. R5/X4
3. R5
4. R5/X4
5. R5
6. R5/X4

NP: HIV negative plasma

Co-Receptor Tropism	# of isolates
X4	15
R5	24
X4/R5	15
Undefined	35
Envelope Subtype	**# of isolate**
Clade A	2
Clade B	76
Clade C	7
Clade D	1
Clade E	3

Figure 7

3. Target Cell Receptor and Co-Receptor Expression

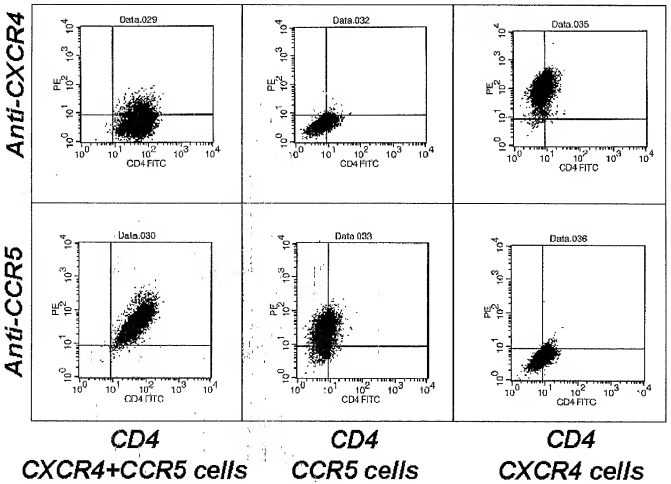
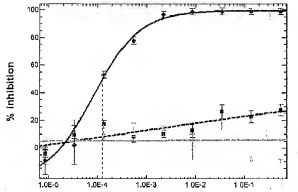
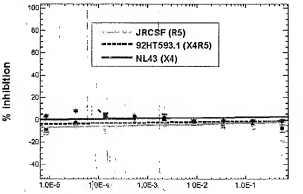
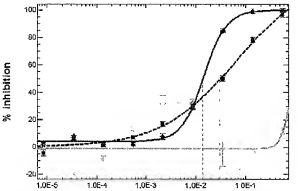


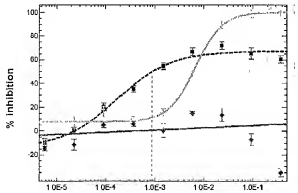
Figure 8

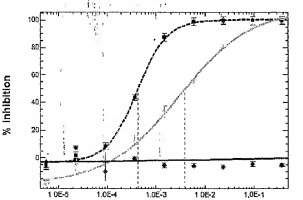
4. **Inhibition By Co-Receptor Antagonists**

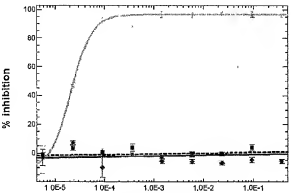












6. Inhibition By Membrane Fusion Inhibitor

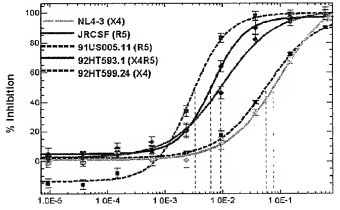


Figure 4A

Fusion Inhibitor Peptides

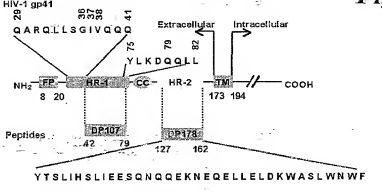


Figure 9

Rimsky, et al., J. Virol. 72 (2):986-993

HIV-1 Site Directed Mutants

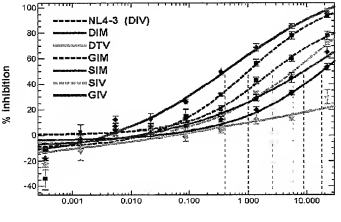


Figure 4B

SDM Virus	DP 178 Sens.[a]	Fold Change[b]
HXB2 G I V		1.0
NL4-3 G I V	S	5.2
NL4-3 D I V	S	12.8
NL4-3 S I V	S	74.2
NL4-3 G I M	S	33.0
NL4-3 D I M	R	113.0
NL4-3 S I M	R	227.4
NL4-3 D T V	R	>281.8
JRCSF G I V		2.1
JRCSF D I V		104.0

[a] Rimsky et al., J. Virol. 72(2):986-993
[b] Fold change in IC50 (vs. HXB2) using PhenoSense HIV Entry Assay